THE GENETIC CONSEQUENCES OF HABITAT FRAGMENTATION[1]

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ABSTRACT

The natural habitats of many species have become fragmented into small "islands," principally by human activities. In this paper we discuss the long-term genetic and evolutionary consequences of fragmentation as inferred from studies on populations that have undergone natural habitat fragmentation in the Ozark Mountains. The Ozarks are the highest land formation found in the midwestern United States. Because of the absence of major geographical barriers around the Ozarks, plants and animals from diverse parts of the continent have been able to invade the area during post-Pleistocene climatic periods. Many of these invasions were short-lived, but the geological and topographical complexity of the Ozarks provided numerous relictual habitats. As a consequence, natural habitat fragmentation occurred for many species, and the fragmentation has often persisted for thousands of years. The genetic and ecological consequences of habitat fragmentation depend critically upon whether or not habitat fragmentation results in a complete cessation of dispersal between the habitat islands. If habitat fragmentation results in the complete genetic isolation of habitat islands, then each "island" becomes demographically independent and local extinction can occur. When there is no opportunity for recolonization, an "extinction ratchet" is possible in which each local extinction brings the global population irreversibly one step closer to total extinction. It is therefore critical to know if habitat fragmentation actually prevents dispersal or not. Unfortunately, studying dispersal patterns directly is usually not feasible. We show how genetic surveys can be used to answer this question. Given demographic fragmentation, we also show how genetic surveys can pinpoint species at high risk for local extinction. These suffer the most severe genetic consequences from habitat fragmentation, such as a drastic loss of genetic variability within habitat islands and inbreeding depression. On the positive side, the genetic variation of a fragmented species is not totally lost but is often present as fixed differences between different local populations. Indeed, a fragmented population is subject to less global loss of genetic variation than an equally sized panmictic population. Consequently, as long as the rate of local extinction is relatively small or counteracted by a recolonization program, a fragmented species can preserve almost all of its genetic variation at the global level for long periods of time. We discuss the optimal design for a recolonization program to prevent global extinction and to maintain high levels of global genetic variation.

As human use of the environment expands, the amount of habitat available for natural communities decreases and the remaining habitat becomes increasingly fragmented. That is, habitats become subdivided into "habitat islands" surrounded by different, usually human-altered, environments. Many tropical and temperate habitats have already been extensively fragmented, and the amount of fragmentation will increase substantially in the foreseeable future.

Conservation biologists are concerned about habitat fragmentation because of its potential to increase extinction rates as predicted by island biogeographic theory (MacArthur & Wilson, 1967), because of "Allee" and "edge" effects, and because of its potential to erode genetic variation through genetic drift in small populations and to promote inbreeding depression. Accordingly, there have been several studies on the impact of habitat fragmentation, but most of these have focused on recent, human-induced habitat fragmentations (e.g., Soulé et al., 1988). Because conservation biology is concerned with the long-term maintenance of biodiversity, we must deal with the long-term ecological and genetic consequences of habitat fragmentation as well as the short-term ones. Once we have a clearer idea of what these long-term consequences are, we can address the question of how best to manage species and communities in fragmented habitats in order to preserve biodiversity for thousands of years.

This paper will focus on the long-term (thousands of years) consequences of habitat fragmentation. In order to study this problem, we cannot use

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recent, human-induced, fragmented habitats. Instead we must look for natural cases of habitat fragmentation that have occurred over the last several thousands of years.

The Ozarks as a Natural Laboratory of Habitat Fragmentation

The Ozarks are the highest elevated land mass between the Appalachian and Rocky mountains in North America. The Ozarks are located primarily in southern Missouri and northern Arkansas, and extend into southern Illinois and eastern Oklahoma. Four features make the Ozarks an ideal natural laboratory for study of the long-term consequences of habitat fragmentation.

The first feature, geological complexity, provides a mosaic of substrates for natural communities. About 1.5 billion years ago, several volcanoes were formed above a hot-spot in what are now known as the St. Francois Mountains in southeastern Missouri (Kisvarsanyi, 1980). Subsequently, this area was periodically invaded by shallow seas, which were associated with the deposition of various sedimentary strata, such as limestone and sandstone. As a consequence, the Ozark area has a complex mixture of igneous and sedimentary rocks, all of which are currently exposed at certain locations.

The second feature is topographical complexity. After the creation of an extensive plateau by sedimentary deposits, the Ozark region has been subjected to erosion by surface and underground streams. The surface topography of the Ozarks was thus transformed from a plateau into a series of ridges and valleys through which flow numerous streams, and which are interspersed by karst features, such as sinkholes and collapsed cave systems. This topographical complexity coupled with the geological complexity mentioned above create much potential habitat diversity in terms of soil types, drainages, and microclimates.

The third feature is the extensive climatic changes that have occurred in this region during and after the Pleistocene Epoch. From about 18,000 to 12,000 B.P. (before present), a boreal spruce forest existed in this area, only to be replaced by a predominantly oak-hickory forest. However, during the Ipsothermal Maximum of 8,000 to 4,000 B.P., the area was hotter and drier than at present, and the oak-hickory forest retreated and was replaced by prairies and deserts. At the end of the Ipsothermal Maximum, the oak-hickory forest reinvaded as the climate became cooler and wetter (COHMAP Members, 1988).

During these climatic fluctuations, plant and animal communities were free to move in and out of the Ozarks because of its fourth critical feature: the absence of major barriers to dispersal around the Ozarks. Many diverse organisms were able to enter the Ozarks during climatically favorable periods, only to be isolated from their main distribution ranges when the climate changed again. Because of the great potential for habitat diversity, relictual populations of many of these species were able to survive in the Ozarks. These relictual populations are found in naturally fragmented habitats that have been cut off from the remainder of their species distribution for a few to several thousands of years. We have been examining several species with relictual Ozark distributions for our studies on habitat fragmentation.

Materials and Methods

Protein electrophoresis was carried out using horizontal starch gel electrophoresis and the methods outlined in Selander et al. (1971) and Thompson & Sites (1986). Gels were prepared by mixing equal volumes of Connaught hydrolyzed starch and Electrostarch, and diluting with gel buffer to a final concentration of 12%.

DNA was isolated as described in Hillis & Davis (1986). This procedure isolates total cellular DNA and thus contains mtDNA and nuclear rDNA. The cellular DNA extracts were digested with various restriction endonucleases using the reaction conditions recommended by the supplier. Agarose gel electrophoresis, Southern blotting, and subsequent hybridizations were carried out as described by Hillis & Davis (1986).

Cryptobranchus alleganiensis. Hellbenders were collected by lifting large rocks and grabbing by hand. Animals were anaesthetized in an aqueous solution of ethyl m-aminobenzoate methanesulfonate, and blood was collected from an incision in the tip of the tail. Blood samples were diluted in a buffer (0.1 M NaCl, 0.05 M tris, 0.001 M ETDA, pH 7.5) and immediately frozen in liquid nitrogen. All hellbenders were released after regaining consciousness except for one or two voucher specimens per river. Blood samples were stored at −80°C until DNA was extracted. Different fractions of the extracted DNA were digested with the following enzymes: BamHI, BclI, BglII, BstEII, DraI, EcoRI, EcoRV, NcoI, PstI, PvuII, SacI, StuI, XbaI, and XmnI. The mtDNA was probed with two homologous clones that contain all but 3.6 kb of the hellbender mitochondrial genome as well as a clone of the entire mtDNA of the frog *Xenopus laevis* (pX131, kindly donated by Dr. Igor Dawid).

Crotaphytus collaris. Collared lizards were collected by hand or by noosing. At the beginning of our work with this species, it was necessary to sacrifice the animals to obtain genetic data. To perform the isozyme survey, 24 animals were collected from across Missouri. Heart, liver, blood, and kidney tissues were used for the isozyme analysis, and skeletal muscle was used for DNA isolation. After this initial survey, all subsequent genetic data were collected from blood samples obtained nondestructively. Blood was collected by piercing the suborbital sinus with a heparinized capillary tube. The blood used for protein electrophoresis was kept on ice until returned to the laboratory. There, the blood was centrifuged for one minute in a hematocrit centrifuge to separate the serum from the red blood cells. The serum was added to an equal volume of distilled water and then frozen for later electrophoresis. The serum was used to score for three different nonspecific esterase loci, two leucine aminopeptidase loci (E.C. 3.4.11.1), two superoxide dismutase loci (E.C. 1.15.1.1), and three general protein loci. The red blood cells were lysed in 1.5 volumes of distilled water and then frozen for later electrophoresis. The red cell gels were scored for an additional esterase locus, alpha and beta hemoglobin, two lactate dehydrogenase loci (E.C. 1.1.1.27), and malate dehydrogenase (E.C. 1.1.1.37).

Blood used for DNA extraction was diluted in buffer and kept either on ice or frozen in liquid nitrogen until returned to the laboratory, where it was stored at −80°C. The extracted DNA used for the rDNA and mtDNA studies was digested with ApaI, BamHI, BclI, BglI, BglII, BstEII, DraI, EcoRI, EcoRV, HincII, HindIII, KpnI, PstI, PvuII, SacI, SacII, StuI, XbaI, and XmnI. The mtDNA was probed with nick translated *Crotaphytus collaris* mtDNA prepared from intact circular mitochondrial DNA isolated from the 24 sacrificed animals by the methods of Lansman et al. (1981). The rDNA was probed with two clones that contained the mouse 18S gene (pFM84) and the mouse 28S gene (PI19), both kindly provided by Dr. Norman Arnheim. Cellular DNA for the analysis of hypervariable VNTR (variable number of tandem repeat) loci ("DNA fingerprinting") was digested with HaeIII and probed with a subclone of the human clone 33.15, kindly donated by Dr. A. Jeffreys.

Trimerotropis saxatalis. Lichen grasshoppers were caught with nets and placed in a cool container until returned to the laboratory, where they were stored at −80°C until being processed for electrophoresis and DNA analysis. Processing involved removing the digestive tract and grinding the internal tissue of the thorax in buffer or distilled water (for the protein electrophoresis work). The only results reported in this paper deal with the enzyme leucine aminopeptidase (LAP) and mtDNA digested with EcoRI.

Results and Discussion

Cryptobranchus alleganiensis

The numerous spring-fed streams that drain the Ozarks provide a habitat for the largest North American salamander, *Cryptobranchus alleganiensis*, commonly known as the hellbender. With the exception of the Ozark populations, hellbenders are found only in the rivers that drain the Appalachians. Consequently, the Ozark hellbenders represent relictual invaders from the eastern United States. Within the Ozarks, hellbenders are distributed into two disjunct populations. One population inhabits the rivers that drain to the north from the Ozarks and empty into the Missouri River or into the Mississippi River close to its confluence with the Missouri River. Members of this population are classified into the subspecies *C. a. alleganiensis*, the same subspecies found in the eastern United States. The other Ozark population inhabits the rivers that drain to the south from the Ozarks and eventually empty into the Mississippi River to the south of the state of Missouri. These southern Ozark animals belong to their own subspecies, *C. a. bishopi*.

The hellbender is totally aquatic and is incapable of terrestrial dispersal. As a result, not only are the two Ozark populations disjunct from one another and their eastern ancestors, but the two Ozark populations are further fragmented into different river systems within these two drainages. Even within a river, their distibution is very patchy. Adult hellbenders are usually found only in those portions of the rivers that have large, loose rocks on the bottom. Unfortunately, virtually nothing is known about the distribution and dispersal behavior of the larvae, although the adults are quite sedentary (Nickerson & Mays, 1973).

In order to infer the pattern of gene flow in this species, we overlaid genetic surveys onto the geographical distribution of the species. Previous studies (Merkle et al., 1977) using isozyme markers revealed that all hellbenders with a few minor exceptions were monomorphic for the same alleles regardless of capture site. Hence there is simply insufficient genetic resolution with isozymes to address the issue of gene flow. We therefore used

TABLE 1. Variable mitochondrial DNA haplotypes found in various populations of *Cryptobranchus alleganiensis* from Missouri and Tennessee. The first eleven variable sites have been mapped on the mitochondrial genome; a "1" refers to the presence of that site and a "0" its absence. The last three restriction fragment length polymorphisms have not yet been mapped, so small letters refer to distinguishable fragment length patterns.

Restriction sites	Haplotypes							
	A	B	C	D	E	F	G	H
BamH1-b	1	0	0	0	0	0	0	0
BglII-d	1	1	0	1	1	1	1	1
BstEII-b	1	1	0	0	0	0	0	0
EcoRV-b	1	1	1	1	1	0	1	1
-c	1	1	0	0	0	0	0	0
-d	1	1	0	0	0	0	0	0
Stu1-5	1	1	0	0	0	0	0	0
-c	1	1	1	1	1	1	1	0
Xmn1-c	1	1	1	1	0	0	0	0
-d	0	0	1	0	0	0	0	0
-e	0	0	0	0	0	0	1	0
Bcl1 Pattern	a	a	b	b	c	c	c	c
Nco1 Pattern	a	a	b	c	a	a	a	a
Xba1 Pattern	a	a	b	b	b	b	b	b

restriction site mapping of mitochondrial DNA (mtDNA) because, at least in mammals, it evolves about 10 times faster than nuclear DNA and accordingly has much higher levels of polymorphism (Brown et al., 1982). As shown in Table 1, eight distinct mitochondrial haplotypes were found in hellbenders collected in Missouri and in Tennessee.

Table 2 shows the geographical distribution of these variants within hellbenders, and Figure 1 portrays these data graphically. Figure 1 also gives a maxium parsimony unrooted cladogram of the eight haplotypes (with a consistency index of 1) as determined by hand. As evident in Figure 1, there is no overlap in the haplotypes found in Tennessee with those found in Missouri. We therefore conclude that the Missouri population is isolated completely from the eastern portion of the range of the species. Moreover, there are no shared haplotypes between the northern and southern drainages of the Ozarks, and as the cladogram shows, the northern and southern haplotypes correspond to distinct branches that are well separated from each other by seven substitutional events. This implies that there is no gene flow between the northern and southern drainages within the Ozarks. Figure 1 also implies that there is restricted gene flow between some of the rivers within the southern and northern drainages. The two southern rivers are fixed for two different haplotypes that define a single branch in the cladogram but that are separated from each other by four substitutional events. This pattern indicates that there is no gene flow between these rivers. In the north, the Niangua River populations are fixed for the B haplotype that is found in only low frequencies in the other northern rivers. However, there are no statistically significant differences in haplotype frequencies among any of the other northern sites, so the possibility of some gene exchange among the Gascanade, Big Piney, and Meramec rivers cannot be excluded. Data from three rivers with multiple collecting sites show no significant differences among sites within a river. This suggests that the patchy distribution of adults within a river does not cause genetic isolation.

In summary, the pattern shown in Figure 1 implies that Missouri populations are genetically isolated from the eastern species range and that northern and southern Ozark drainages are isolated from one another. In addition, many rivers define genetically isolated populations from nearby rivers, but local populations within a river are not genetically isolated from one another.

Hence, by overlaying the genetic survey results upon the geographical distribution of the populations, we have been able to infer much about when habitat fragmentation results in genetic isolation and when it does not for this species. This conclusion would be virtually impossible to make from traditional dispersal studies because of the extreme difficulty of following the fate of larvae in this species and because the adults are extremely long-lived, which invalidates the use of short-term studies. These problems are not limited to hellbenders and occur frequently with other species. For example, one of the better dispersal studies ever done with amphibians involved marking over 25,000 tadpoles and metamorphosing Fowler's toads, which after five years of work resulted in the recapture of 37 adults (Breden, 1987). However, given sufficient genetic resolution, the same types of inferences about genetic subdivision can be made from a genetic survey of a much smaller number of adults.

We believe that using genetic surveys to infer when habitat fragmentation results in complete genetic isolation and when it does not represents one of the most important applications of genetics to the problem of habitat fragmentation. The reason for this importance lies in the fact that the long-term ecological and genetic fate of a fragmented population depends critically upon whether or not there is genetic isolation between the habitat islands, as will be illustrated by our next example.

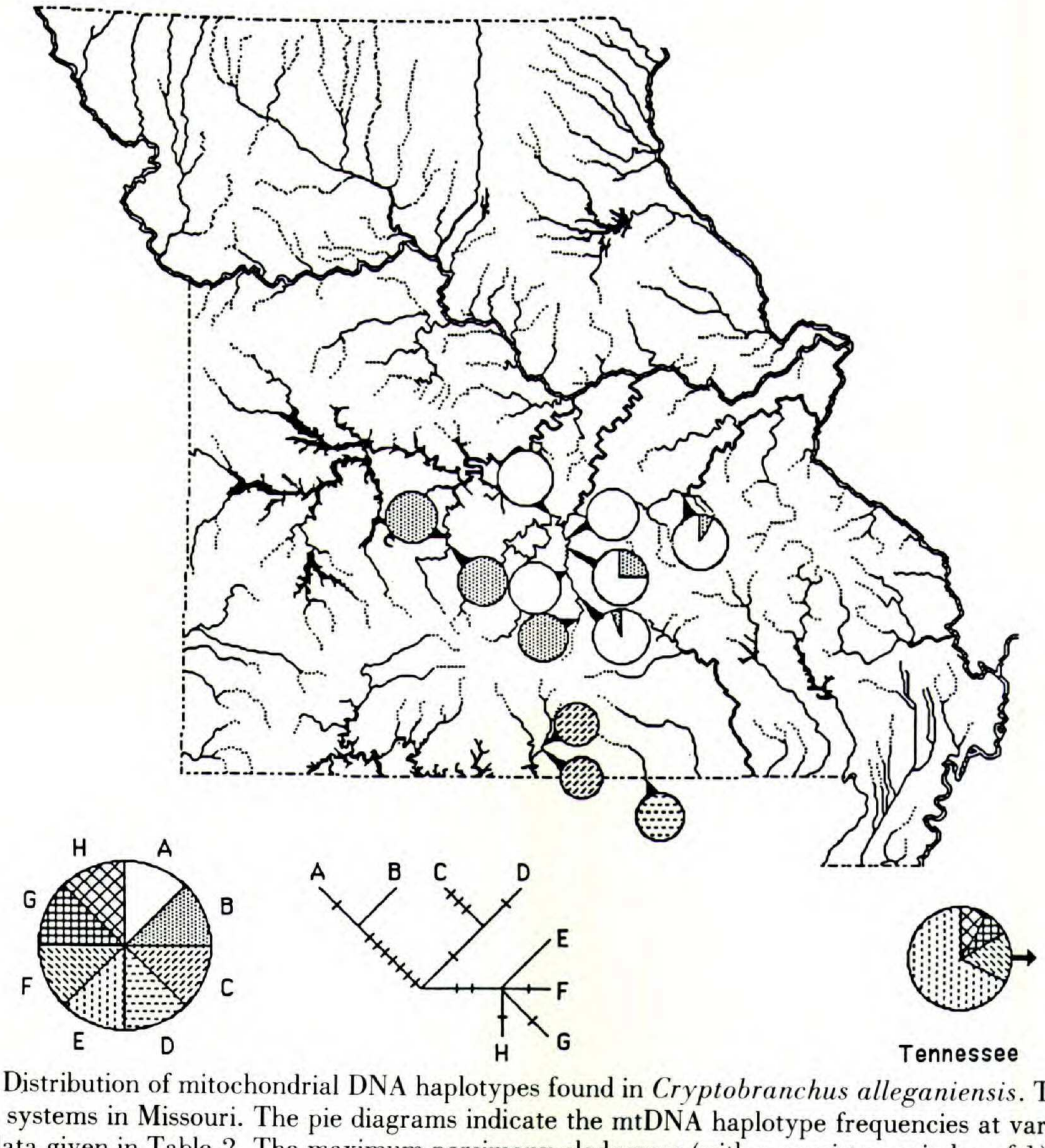

FIGURE 1. Distribution of mitochondrial DNA haplotypes found in *Cryptobranchus alleganiensis*. The map shows the major river systems in Missouri. The pie diagrams indicate the mtDNA haplotype frequencies at various collecting sites from the data given in Table 2. The maximum parsimony cladogram (with a consistency index of 1) that connects the various haplotypes is shown at the bottom.

CROTAPHYTUS COLLARIS

The main range of collared lizards is in the southwestern United States and in northern Mexico. They also reside in highly fragmented populations scattered thoughout the Ozarks. These desert animals most likely invaded Missouri during the Ipsothermal Maximum of 8,000 to 4,000 years B.P. With the reinvasion of the oak-hickory forest around 4,000 B.P., these lizards were only able to survive in Missouri in relictual, desertlike habitats known as glades. Glades form on exposed outcrops of sedimentary or igneous rock, usually near tops of ridges with southerly or southwesterly exposures. The poor, rocky soil of glades inhibits tree growth, and the rocky, southerly exposures create a very dry, hot microhabitat. As a consequence, a large number of animals and plants normally found in deserts or dry prairies are also found in Ozark glades, including collared lizards.

Glades vary in size from less than an acre up to several hundreds of acres (Nelson & Ladd, 1981). The larger glades tend to be on dolomite and have a more prairielike flora and fauna than the more desertlike igneous glades. Collared lizards are one of the few glade endemics that can inhabit both of these major glade types. The dolomitic glades are also more susceptible to forest invasion, and with the advent of forest fire control in historic times, there has been a secondary phase of fragmentation of the dolomitic glades due to forest encroachment (Beilmann & Brenner, 1951). Consequently, with the collared lizards, a primary phase of habitat fragmentation occurred 4,000 years ago followed by a secondary phase on dolomitic glades within the last two centuries.

As with the hellbenders, we can use genetic surveys to infer when habitat fragmentation results in genetic isolation. Table 3 summarizes the results of genetic surveys using protein electrophoresis on 17 loci and restriction mapping of nuclear ribosomal DNA (rDNA) and mtDNA using 19 restriction enzymes. The amounts of genetic variation found with any one of these techniques are modest, with the discovery of only one polymorphic protein locus and four haplotypes each for the rDNA and

TABLE 2. Geographical distribution of mitochondrial DNA haplotypes found in various populations of *Cryptobranchus alleganiensis* from Missouri and Tennessee. The haplotype designations are given in Table 1.

Locations	Numbers of animals with haplotypes: A	B	C	D	E	F	G	H
Northern Missouri								
Big Piney River								
Barton Branch	0	1	0	0	0	0	0	0
Boiling Spring	23	1	0	0	0	0	0	0
Slabtown Spring	11	0	0	0	0	0	0	0
Spring Creek	9	3	0	0	0	0	0	0
Devils Elbow	11	0	0	0	0	0	0	0
Gasconade River	12	0	0	0	0	0	0	0
Niangua River								
Site 1	0	14	0	0	0	0	0	0
Site 2	0	3	0	0	0	0	0	0
Meramec River	21	2	0	0	0	0	0	0
Southern Missouri								
North Fork of White River								
Site 1	0	0	14	0	0	0	0	0
Site 2	0	0	3	0	0	0	0	0
Spring River	0	0	0	6	0	0	0	0
Tennessee								
Little River	0	0	0	0	9	2	1	0
Beaverdam Creek	0	0	0	0	7	0	0	1

mtDNA (Table 3). Although no one genetic system detects much variation, the pooled systems provide a reasonable degree of genetic resolution, as shown in Figure 2. The joint pattern strongly suggests that different glades are genetically isolated from one another. As Figure 2 shows, almost all of the genetic variation is found as between-glade differences.

Strong genetic differences can exist on even a very local scale. For example, the lizards on Mina Sauk glade are fixed for rDNA and mtDNA haplotypes different from those for which the lizards on Proffit Mountain are fixed, despite the fact that these two areas are only about eight miles apart as one would walk along ridge tops (the most probable dispersal route for lizards). Even on Proffit Mountain, there seems to be strong genetic differentiation between glades only a quarter of a mile from one another, although small sample sizes make this a tentative conclusion.

There thus seems to be extreme genetic fragmentation in the northern Ozarks. There is likewise evidence for extreme genetic fragmentation in southwestern Missouri. The populations on Dewey Bald, Hercules Glade, and Glade Top Trail are all fixed or nearly fixed for different genotypes. The only significant geographical pattern is that the three glade populations on Glade Top Trail all share a unique genotype. Since these three dolomitic glades were undoubtedly a single glade before forest fire control this genetic sharing is not surprising. Moreover, the Lookout population on Glade Top Trail is the only polymorphic population for these genetic systems found on a natural glade (as will be discussed shortly, the Proffit Mountain population is not inhabiting a natural glade). This observation supports the hypothesis that the Glade Top Trail populations were a single, large population until very recently.

As Figure 2 summarizes, the glades isolated at the end of the Ipsothermal Maximum display a genetic pattern that suggests complete genetic isolation. The lack of any clear geographical pattern to how this between-glade variation is distributed indicates that the isolation at the end of the Ipsothermal Maximum was very rapid; that is, all the glades became isolated from one another more or less at the same time, so that there is no evidence for the pattern expected under an isolation by distance model.

Once again, we have an example of how genetic surveys can be used to determine the extent to which habitat fragmentation causes genetic fragmentation. In the case of the collared lizards, this genetic fragmentation appears to be more severe than it is for the hellbenders. The primary ecological consequence of complete genetic isolation is demographic fragmentation. By this, we mean that the dynamics of population growth, age structure, etc., within a habitat island exerts no direct influence upon the comparable demographic variables in other habitat islands. Of course, the demographic states among habitat islands could be correlated because of a dependence upon some global environmental state, but there are no direct population-level interactions between habitat islands. Also, with no genetic interchange, evolution proceeds independently within each habitat island except for indirect correlations caused by global environmental conditions.

If the demographically fragmented demes are small in size, genetic drift becomes an important evolutionary force. Ultimately, genetic drift is expected to result in the loss of genetic variation. The rate at which this loss occurs depends upon the variance effective breeding size of the fragmented subpopulations. As can be seen from Figure 2, most glade populations of collared lizards show

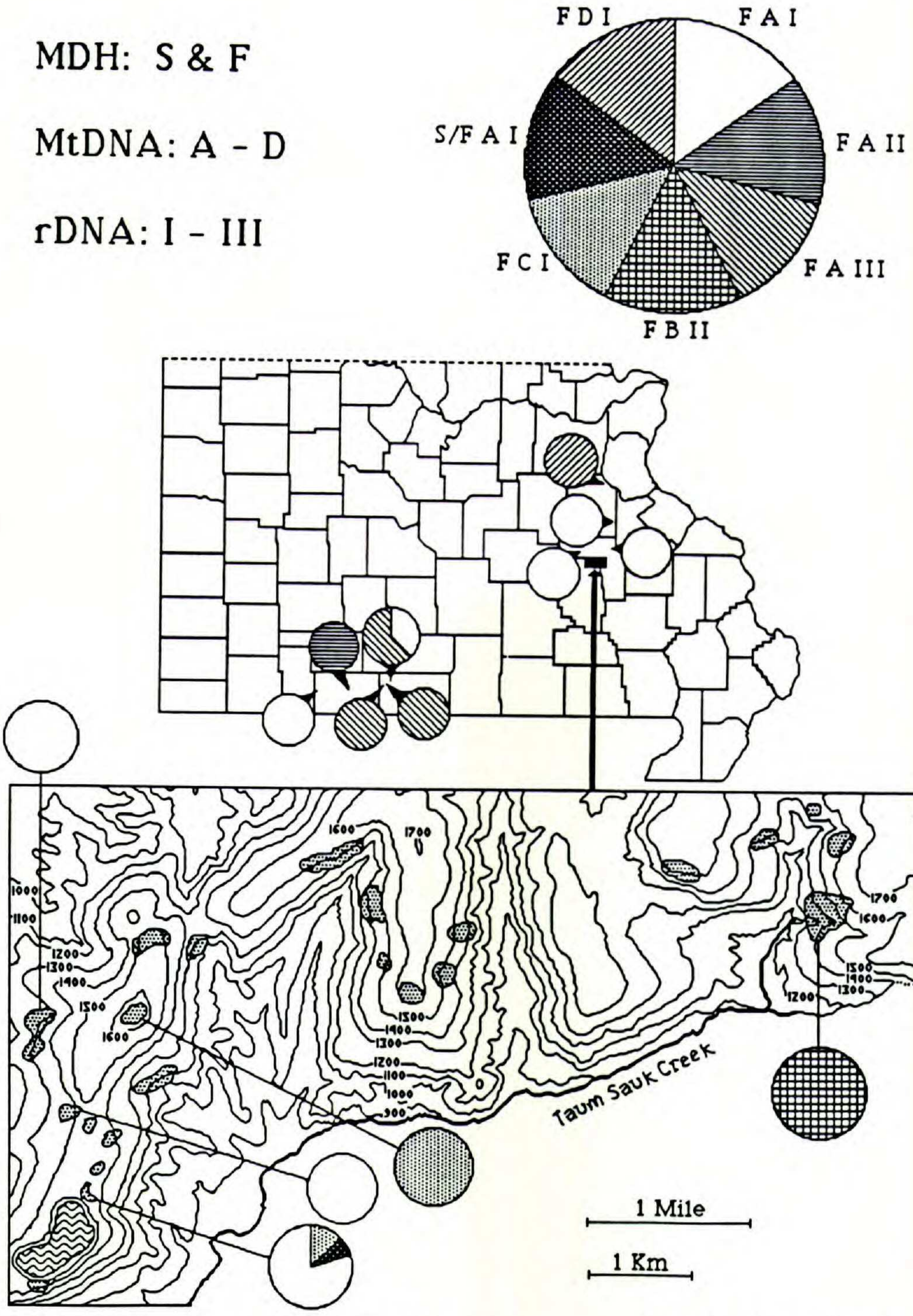

FIGURE 2. Distribution of isoyme, mtDNA, and rDNA genetic variants found in *Crotaphytus collaris*. A county outline map of the southern half of Missouri is shown, with pie diagrams indicating the genotype frequencies at various collecting sites from the data given in Table 3. An expanded scale map is shown of the area between Taum Sauk Mountain (on the far right of the expanded map) and Proffit Mountain (on the far left). Glades are indicated by stippled areas on this map. Contour intervals are 100 feet.

no polymorphism whatsoever. The only exceptions to this pattern are the Glade Top Trail populations, which were probably fragmented only very recently, and the population found on the parking lot near the Upper Taum Sauk Reservoir on Proffit Mountain. The reservoir has its walls reinforced by loose boulders, and this provides a dispersal corridor for lizards between previously isolated natural glades (indeed, lizards have been observed on these walls). Thus, this population is most likely an amalgam of previously isolated populations. Consequently, we conclude that most genetic variation in these lizards exists as fixed differences between glades rather than as polymorphisms within glades.

Unfortunately, these conclusions are tempered by the small sample sizes of several of these glade populations and by the relatively low levels of overall genetic variation. To obtain a more accurate quantification of the partitioning of genetic variation between and within glades, we performed an additional genetic survey using hypervariable VNTR loci on five glade populations having sample sizes between 4 and 58. We found a total of 13 variable, high-molecular-weight bands that could be reliably

TABLE 3. Results of various genetic surveys on *Crotaphytus collaris* populations found in Missouri glades and in central and western Oklahoma. The only polymorphic enzyme locus, malate dehydrogenase (MDH), had two alleles: "fast" (F) and "slow" (S). Four mtDNA haplotypes (designated A through D) were discovered, all due to length variation scorable with BclI digests. Four rDNA haplotypes were discovered as defined by the presence (+) or absence (−) of a PvuII restriction site and by length variants in the nontranscribed spacer detected by BamHI and by EcoRI. The most common haplotype is designated as I, and is coded as: −−− (i.e., it lacks the PvuII site, lacks the BamHI length variant, and lacks the EcoRI length variant). The other haplotypes are: II (+−−), III (++−), and IV (−−+).

Populations	Sample sizes	MDH	mtDNA	rDNA
Highway 21 Glade	3	F/F	D	I
Highway M Glade	2	F/F	A	I
Graniteville Quarry	1	F/F	A	I
Mina Sauk Glade	12	F/F	B	II
Proffit Mountain #4	1	F/F	A	I
Proffit Mountain #5	2	F/F	A	I
Proffit Mountain #7	2	F/F	C	I
Proffit Mountain parking lot	9	6: F/F	A	I
		2: F/F	C	I
		1: S/F	A	I
Glade Top Trail saddle	5	F/F	A	III
Glade Top Trail pinnacle	3	F/F	A	III
Glade Top Trail lookout	5	3: F/F	A	III
		2: F/F	A	I
Dewey Bald	2	F/F	A	II
Hercules Glade	2	F/F	A	I
Central Oklahoma	3	F/F	A	IV
Western Oklahoma	1	F/F	A	II

scored (there was obvious variation at some low-molecular-weight bands, but it was difficult to score). The results are summarized in Figure 3. As can be seen, a total of 25 different band phenotypes are observed, but only four of these phenotypes are found in more than one glade. We quantified the extent of partitioning of variation at the VNTR loci by estimating the proportion of shared bands within glades ($P_w = 0.74$) and between glades ($P_b = 0.50$). Moreover, we estimated the correlation of band phenotypes within glades relative to the total population to be 0.47. This correlation should be proportional to the standard F_{st} statistic (Rothman et al., 1974), which unfortunately cannot be estimated directly because the bands must be regarded only as phenotypes and not genotypes in the absence of information about number of VNTR loci and band homologies. However, this correlation will be greater than 0 only if F_{st} is greater than zero. This correlation of 0.47 is significantly different from 0 at the 1% level. Hence, F_{st} is also significantly different from 0 and rather large in value. Once again, we conclude that the majority of genetic variation is found as between-glade differences.

The fact that most genetic variation is found between glades not only confirms our conclusion that glades are demographically independent from one another, but it also implies that the variance effective breeding sizes within glades are very small. This inference is consistent with other data. The population on Sandy Ridge in Jefferson County seems to be one of the largest in the northeastern Ozarks. Yet the sample of 58 lizards constitutes a virtual census. Dr. Owen Sexton (pers. comm.) has followed this population in detail and has found that the adult population during the breeding season fluctuated between 21 and 79 individuals 1975–1985. Such small population sizes would cause a rapid partitioning of the ancestral genetic variation into the between-glade component.

These small population sizes imply that the glade populations should be very prone to local extinction. When populations are this small, inbreeding depression, environmental fluctuations, and demographic stochasticity can greatly increase the probability of local extinction (Quinn & Hastings, 1987). Given that there is no dispersal between glades, an "extinction ratchet" operates in which each local extinction brings the total population one step closer to global extinction. It is important to note that the rate at which this extinction ratchet operates is primarily a function of local, not global, population size. Hence, populations that are both

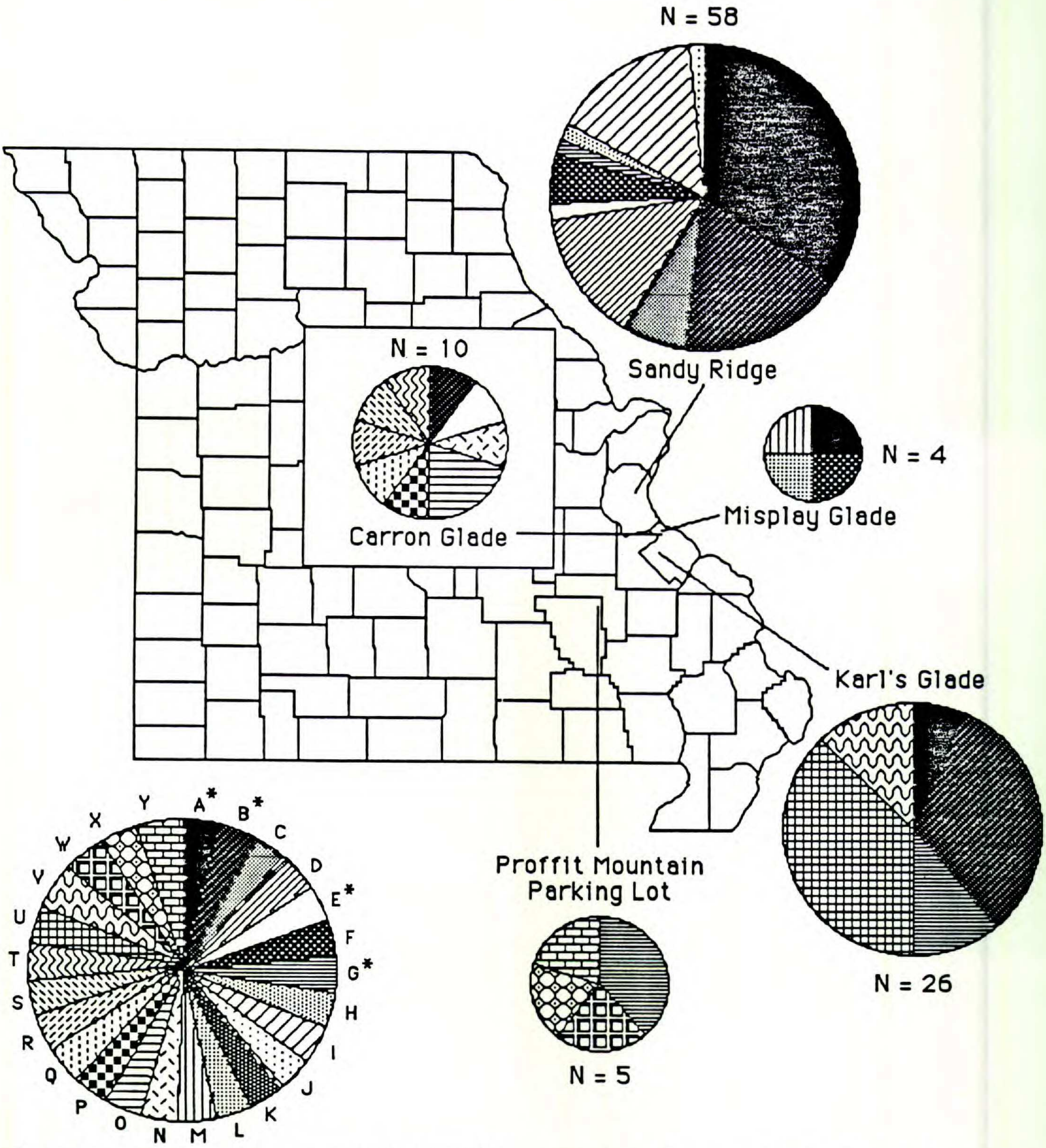

FIGURE 3. Distribution of hypervariable VNTR ("DNA fingerprinting") phenotypes found in *Crotaphytus collaris*. A county outline map of Missouri is shown, with pie diagrams indicating the phenotype frequencies in five glade populations. The number above, alongside, or below each pie diagram is the sample size from that glade. The DNA was cut with HaeIII and probed with the human fingergprinting clone 33.15.

fragmented and small should have a very high rate of local extinction and therefore high probabilities of global extinction.

There is much circumstantial evidence for local extinction in the lizards. The recorded county distribution for collared lizards in Missouri (Johnson, 1987) excludes many counties that have excellent glade habitat (Nelson & Ladd, 1981) and that are adjacent to counties known to have collared lizards. There is no apparent reason why the lizards should be absent from these counties, especially in view of the distributions of other glade-inhabiting animals and plants. Even within the counties known to have lizards, the authors have never observed collared lizards on many apparently suitable glades despite several trips to these glades and despite the fact that collared lizards are found on nearby glades. For example, Hawn State Park contains some sandstone glades that appear to be excellent habitat for collared lizards. Just outside the park are several

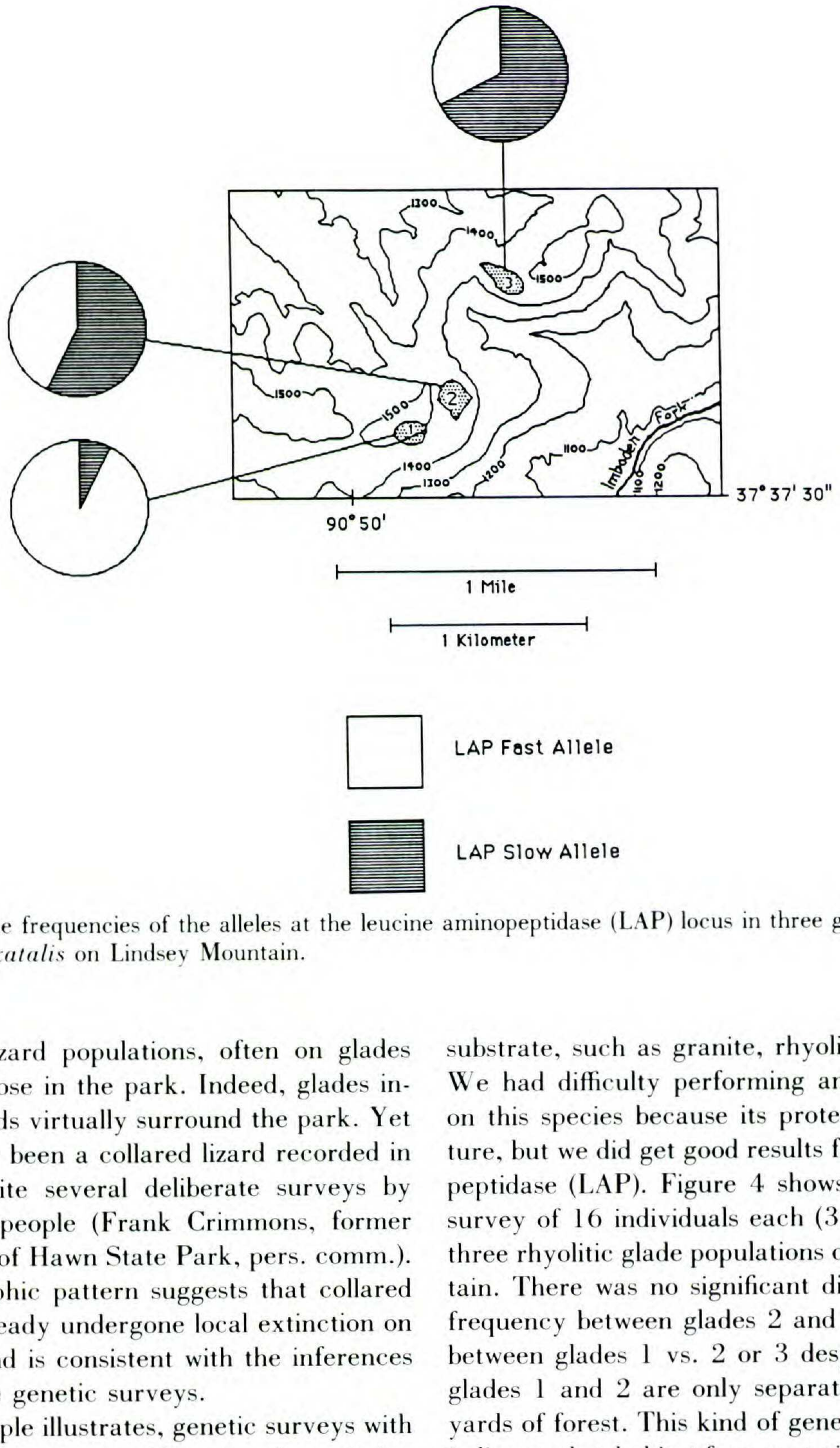

FIGURE 4. The frequencies of the alleles at the leucine aminopeptidase (LAP) locus in three glade populations of *Trimerotropis saxatalis* on Lindsey Mountain.

very healthy lizard populations, often on glades smaller than those in the park. Indeed, glades inhabited by lizards virtually surround the park. Yet there has never been a collared lizard recorded in the park, despite several deliberate surveys by knowledgeable people (Frank Crimmons, former superintendent of Hawn State Park, pers. comm.). This biogeographic pattern suggests that collared lizards have already undergone local extinction on many glades and is consistent with the inferences drawn from the genetic surveys.

As this example illustrates, genetic surveys with sufficient resolution to partition genetic variation between and within habitat islands can quickly identify those species at high risk for local extinction. The collared lizards are an example in which the genetic partitioning implies a high risk for local extinction. Our next example shows how genetic surveys can identify a fragmented species at a low risk for local extinction.

TRIMEROTROPIS SAXATALIS

Another glade inhabitant in the Ozarks is the lichen grasshopper, *Trimerotropis saxatalis*. This species is found primarily on glades with an acidic substrate, such as granite, rhyolite, or sandstone. We had difficulty performing an isozyme survey on this species because its proteins rapidly denature, but we did get good results for leucine aminopeptidase (LAP). Figure 4 shows the results of a survey of 16 individuals each (32 genomes) from three rhyolitic glade populations on Lindsey Mountain. There was no significant difference in allele frequency between glades 2 and 3, but there was between glades 1 vs. 2 or 3 despite the fact that glades 1 and 2 are only separated by about 150 yards of forest. This kind of genetic differentiation indicates that habitat fragmentation once again resulted in genetic isolation. Also note from Figure 4 that all three populations are polymorphic for the same LAP alleles. We are currently surveying for genetic variability in rDNA and mtDNA with restriction enzymes. Both DNA systems have revealed much genetic variation, but this variation has yet to be mapped. However, it is obvious from the Southern blot patterns that there is extensive polymorphism within all glade populations and that many variants are shared with other widely scattered glades—a pattern that is in great contrast to that observed for the collared lizards. Never-

TABLE 4. Distribution of mtDNA variants in four glade populations of *Trimerotropis saxatalis*. Cutting mtDNA with EcoRI yields five distinct patterns in these populations, labeled A–E.

	Glades			
Haplotypes	Sandy Ridge	Russell Mountain—2	Proffit Mountain—12	Steagle Mountain—7
A	2	3	3	0
B	4	3	6	6
C	0	0	0	2
D	1	3	0	1
E	0	2	0	0

theless, glade populations will frequently have at least some genetic variants not found in other nearby populations, which indicates absence of gene flow. For example, five distinct restriction fragment-length patterns are visible when the mtDNA is cut with EcoRI. Table 4 shows the number of individuals bearing these five haplotypes in four glades, and Figure 5 shows the geographical locations of these glades. As can be seen, all populations are polymorphic for mtDNA haplotypes, and most haplotypes are found throughout widely scattered areas. Yet, the Russell Mountain, glade 2 population has two mitochondrial haplotypes not found on the nearby (closer than 6 miles or 10 kilometers) Proffit Mountain, glade 12 population—a difference that is significant at the 1% level. Such a pattern is strongly indicative of no gene flow even between geographically close glades.

Although the lichen grasshoppers are highly fragmented into isolated glade populations, the large amount of within-glade polymorphism implies that the numbers within each glade are sufficiently large to insure that genetic drift is weak. This in turn implies that this species is in very little danger of local extinction except from environmental challenges that would cover the entire spatial scale of their habitat. This prediction is consistent with our collecting experiences for this species. Of 85 acidic glades visited that were a quarter of an acre or larger, these grasshoppers were found on all 85. In contrast, collared lizards were only observed on 14 of these 85 glades (however, some of these glades were only visited once, so it is probable that collared lizards are on more than 14 of them). These observations support the inference from the

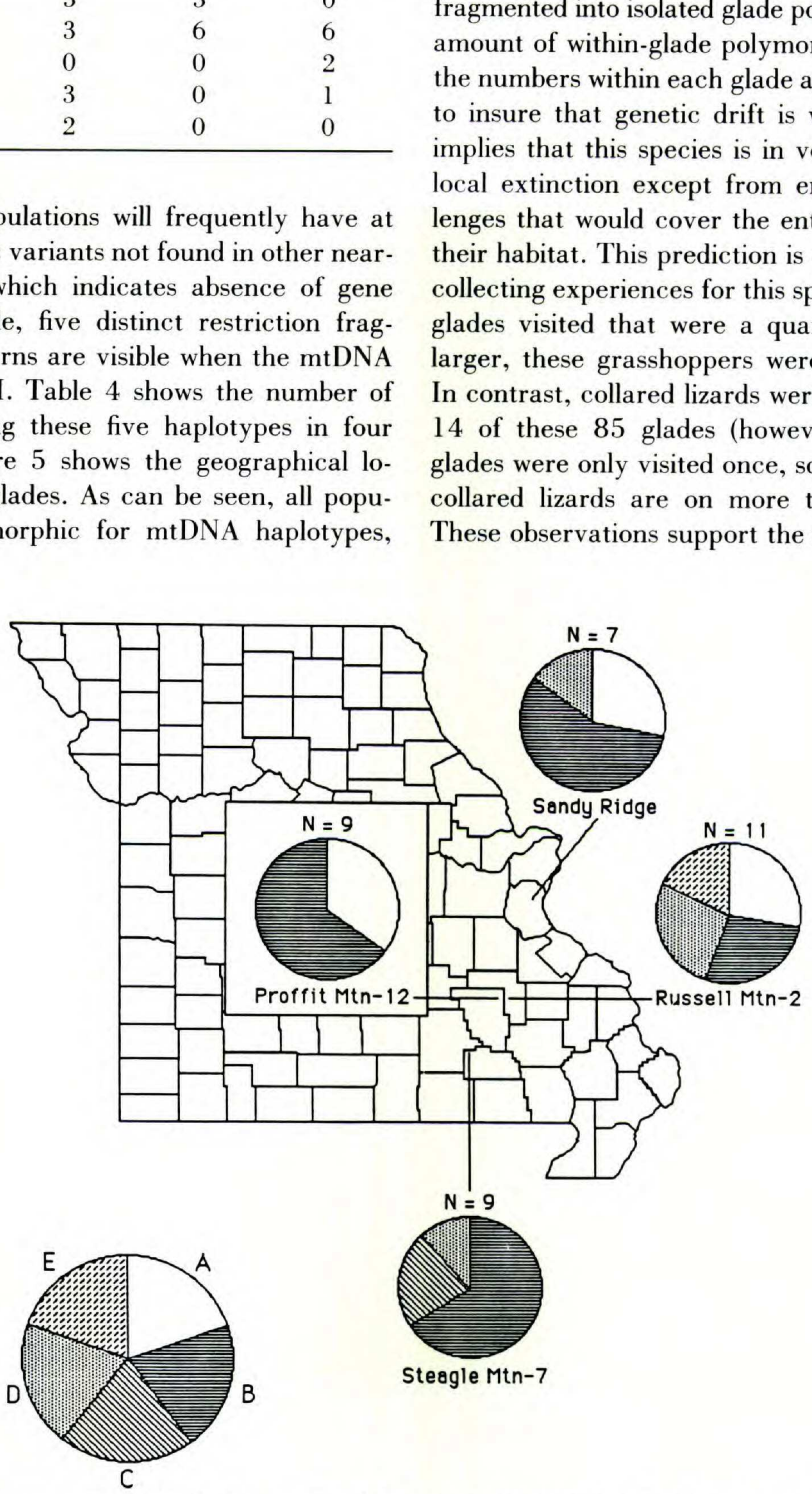

FIGURE 5. Distribution of mtDNA haplotypes found in *Trimerotropis saxatalis*. A county outline map of Missouri is shown, with pie diagrams indicating the haplotype frequencies in four glade populations. The number above each pie diagram is the sample size from that glade.

genetic data that the local populations of lichen grasshoppers are not at high risk for local extinction.

Management Recommendations

The ecological and genetic consequences of habitat fragmentation depend critically upon whether or not there is dispersal between habitat islands. The examples given in this paper show that genetic surveys are an extremely useful tool for addressing this critical question. The importance of genetic surveys is augmented further by the fact that it is not feasible to study dispersal directly for many species, but genetic surveys can be performed on virtually any species. Even when dispersal studies are feasible, genetic surveys are a more accurate means of inferring demographic fragmentation. For example, Lewis (1982) studied dispersal in the white-browed sparrow weaver, *Plocepasser mahali*. These birds are colonial breeders, and Lewis discovered that there is much successful immigration of dispersing birds into the smaller colonies. However, these colonies are not generally reproductively successful, and most birds in them are effectively dead genetically. Almost all successful reproduction is limited to large groups, and there is very little immigration into these. Hence, there is very little gene flow in this species despite much dispersal. On the other hand, basic population genetic theory (Crow & Kimura, 1970) shows that even very low dispersal rates—particularly, rare long-distance dispersal events—can be sufficient to maintain local populations as a single genetic unit. It is virtually impossible to estimate rare, long-distance dispersal rates in most species. Thus, genetic surveys provide a more reliable indicator of gene flow patterns than do dispersal studies. Genetic surveys are therefore a very powerful tool in identifying the pattern of demographic fragmentation caused by habitat fragmentation, and it is recommended that genetic surveys be utilized much more for this purpose.

Genetic surveys are also useful in identifying species at high risk for local extinction, given that demographic fragmentation has occurred. As illustrated by the contrast between collared lizards and lichen grasshoppers, we can make inferences about the long-term effective population sizes within habitat islands by partitioning the genetic variability into within-habitat-island and between-habitat-island components.

Species displaying small effective sizes are at most risk for inbreeding depression, demographic stochasticity, and extinction through environmental fluctuations. Many species that are endangered to begin with have low population sizes. Therefore, the most-endangered species suffer most from demographic fragmentation. Demographic fragmentation can therefore greatly accelerate the rate of extinction of an endangered species through the operation of the extinction ratchet. One obvious method of stopping the advance of the extinction ratchet is to recolonize artificially in order to counteract local extinction. In conjunction with the Missouri Conservation Commission, we have begun such a recolonization program for collared lizards in the Missouri Ozarks in order to study and monitor the success of various release strategies. Because the lizards are long-lived and the release program began in 1984, we do not have sufficient data to make evaluations. Nevertheless, our experiences in designing this release program have general implications.

One of the first decisions to be made is the goal of the release program. We feel that such release programs should preserve the genetic variability of the species while protecting it from extinction. With respect to genetic variation, there is a silver lining in the generally dark cloud of habitat fragmentation. As can be seen from the collared lizard example, when a population is fragmented into small, demographically independent isolates, there is rapid partitioning of the available genetic variation from within-habitat to between-habitat. The important point is that this is just a redistribution of the genetic variation, not its elimination. Genetic variation is still present as fixed differences between local populations. Moreover, population genetic theory (Maruyama, 1972) showed that global genetic variation is maintained more efficiently by a fragmented population than by a panmictic population, given an equal total size. The primary cause of loss of genetic variation in a finite population is genetic drift, but the genetic differences that are fixed between local populations can only be lost by extinction of the entire population.

Local extinction, however, can modify this prediction. As local extinction occurs, not only is population size reduced at the global level, but any unique genetic variants found in that local population are lost as well. As long as there are many local populations available, the loss in global genetic variation caused by local extinction is very little. But as the extinction ratchet decreases the number of local populations, genetic variation loss will accelerate. Accordingly, recolonization intervention is needed to prevent extinction and to insure that a sufficient number of local populations are maintained so that global genetic variation can be preserved. However, the goal of preserving genetic

variation places constraints on how the recolonization should be done.

There are two basic strategies in a recolonization program. One strategy is to obtain all the animals for a release from a single, local, large population. The alternative is to obtain the release animals from several local populations and recolonize with a mixed population. Arguments can be made for both strategies.

With a mixed release, high levels of genetic variability can be reestablished at the local population level. However, since the released population will in general be small, this variation will be rapidly lost due to genetic drift. Nevertheless, even a temporary infusion of genetic variability into the local population can be beneficial. Evolution within the local population can occur only if there is genetic variability, so natural selection in the local population is possible for the first few generations after the release of a mixed population. Hence, a mixed release allows some adaptation to the local environment. This could be important in increasing the chances of success of the released population if the environment in which the release takes place is not identical to the environments experienced by the source populations.

Local adaptation could, however, sometimes favor the strategy of nonmixed release. Frequently, local adaptation in small, isolated populations is achieved by the accumulation of "coadapted" gene complexes (Templeton, 1986). Even populations adapting to the same environment will often achieve that adaptation in genetically distinct and incompatible fashions. When these coadapted complexes are broken down by recombination, the average fitness of the population could be lowered dramatically—a phenomenon known as "outbreeding depression" (Templeton, 1986; Templeton et al., 1986). If severe enough, an outbreeding depression could greatly increase the chances of extinction of the released populations.

Annest & Templeton (1978) have experimentally monitored the evolutionary and ecological significance of outbreeding depression in *Drosophila* populations. They showed that the lowest population sizes occur during the first generation in which recombination can break up coadapted complexes (generally, the F_2 or backcross generations). Therefore, extinction due to outbreeding depression generally will occur in the first few generations after release. After that, selection operates to reestablish one of the parental coadapted gene complexes or evolve a new one. In either case, the absolute fitness increases and population size rises. Annest & Templeton (1978) found that when a new coadapted complex arose, it generally had superior fitness traits to any of the input parental complexes. Thus, if the population can survive the first few generations, the outbreeding depression will be eliminated by the action of natural selection and it is possible that the surviving population will have higher fitness than any parental population. Consequently, even if there is an outbreeding depression, mixed releases still might be best in the long run. It would be essential to monitor the released populations closely for at least the first three to five generations. For example, we obtain a blood sample from all collared lizards before release to provide genetic markers to detect outbreeding and/or inbreeding depressions. These genetic markers will also allow us to estimate accurately the variance and inbreeding effective sizes of the released populations by observing the rate of decay of genetic variation through time. If severe outbreeding depressions occur, the mixed release strategy might have to be abandoned to insure the survival of the populations in the recolonized areas.

In addition to outbreeding depression, the released population may suffer from inbreeding depression. Inbreeding depression is usually caused by the increased incidence of homozygosity for recessive deleterious alleles that occurs with inbreeding. Inbreeding and outbreeding depression are not mutually exclusive since they can involve different genetic systems found in the same organisms. When inbreeding occurs because of small population size, deleterious alleles have a finite chance of going to fixation. Hence, the real danger in small, isolated populations is that the inbreeding depression will be fixed by genetic drift. O'Brien et al. (1985) have argued that such a fixed inbreeding depression may account for the low reproductive performances of cheetahs. Fixed inbreeding depressions represent a serious problem for the survival of local populations and for the species. We would expect the local populations displaying the most severe inbreeding depressions to go extinct more rapidly. Therefore, as time proceeds, the average severity of fixed inbreeding depression should decrease, but over even long periods of time, we expect fixed inbreeding depression to rise again. When population sizes are very small, even deleterious mutations have a finite chance of fixation (Crow & Kimura, 1970), and once fixed, there is no mechanism to purge the deleterious mutation from the local populations under complete genetic isolation. This results in a situation analogous to "Muller's ratchet" in which deleterious alleles will tend to accumulate until the population is driven to extinction (Muller, 1964).

By carrying out a mixed release, the temporary infusion of heterozygosity will alleviate the inbreeding depression for the first few generations after release. As the generations progress, inbreeding will become reestablished in the released populations, and hence inbreeding depression may reappear. However, just as outbreeding depressions can be eliminated by the operation of natural selection, so too can inbreeding depressions (Templeton & Read, 1983, 1984). With a mixed release, natural selection has a renewed opportunity to eliminate deleterious alleles. Random drift may once again cause fixation of deleterious genes, but natural selection biases the stochastic fixation process against that possibility. Also, this temporary influx of genetic variation allows recombination to be effective at producing new genotypes, and this recombination can help undo the damage done by "Muller's ratchet" (Muller, 1964). By releasing from a single source population, there is no opportunity for selection or effective recombination to operate, and therefore any fixed inbreeding depression in the source populations will remain fixed and "Muller's ratchet" will proceed unabated.

The benefits from selective processes operating in released populations increase as the initial amount of genetic variation increases. Accordingly, the best mixed release strategy combines individual populations from a large number of habitat islands. The selective benefits also increase as the number of generations with genetic variation after release increases. The number of genetically variable generations can be maximized by making the released population as close as is practical to the ultimate carrying capacity of the habitat island to be recolonized.

Mixed releases also aid in the goal of preserving overall genetic variability. When all individuals for release are drawn from a single source population, it is essential that the source population be very large so that harvesting individuals from it will not endanger the source as well. Such large releases reduce the chances of extinction and increase the opportunity for natural selection to promote local adaptation and eliminate outbreeding and inbreeding depressions. Only a few source populations will be sufficiently numerous to support this extensive harvesting. For example, we have identified only four glades that could support a harvesting of 10 collared lizards—our minimum release size—without seriously endangering the survival of the source population. As the collared lizard example shows, the released populations under the single source strategy will often be genetic clones of a small number of source populations. Thus, genetic variation at the global level will be rapidly depleted under this recolonization strategy.

In contrast, the multiple-source recolonization strategy can preserve large amounts of global genetic variation. First, because only a few individuals need be taken from any single local population, many more local populations can qualify as source populations, as illustrated by our collared lizard release program. By doing mixed releases in which all animals come from different glades, we need only harvest a single animal from any particular glade. Virtually all natural populations can endure that level of harvesting without ill effect. The recolonization program this way can tap into much more of the global genetic variability contained in the fragmented species. After release, genetic drift will cause loss of variation. However, different releases will undoubtedly become fixed for a different array of genetic variants. Hence, there will be no tendency to "clone" a handful of source populations; instead, the released populations will be genetically diverse relative to one another and to their sources. In this manner, very high levels of global genetic variation can be maintained in the fragmented species despite high rates of local extinction. Follow-up monitoring of the released populations is just as important as the initial release in order to insure that genetic diversity is being preserved and to check for the possibility of outbreeding depressions.

Summary of Recommendations

Genetic surveys are an extremely powerful tool for identifying demographically independent habitat islands and should be used more extensively to infer the pattern of genetic fragmentation. Indeed, genetic surveys offer a more reliable means of inferring demographic fragmentation than dispersal studies and can be applied easily to a wide diversity of organisms.

Second, genetic surveys, particularly those utilizing high-resolution techniques, such as DNA fingerprinting, can be used to spot fragmented species at high risk for local extinction. This is accomplished by quantifying the partitioning of genetic variation between and within demographically independent habitat islands.

For species with a high risk of local extinction, recolonization programs are needed to protect the fragmented species against global extinction and to preserve its pool of genetic diversity. In general, a mixed release strategy is best, with the numbers to be released ideally as close as possible to the carrying capacity. Released populations must be

monitored genetically and demographically for the first few generations after release to detect potential inbreeding and/or outbreeding depression and periodically thereafter to insure that genetic diversity is indeed being preserved.

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